CleanPCR

Catalog Nos. CPCR-0005, CPCR-0050, CPCR-0500
Manual revision v2.01

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Information in this document is subject to change without notice.

Introduction and Principle

The CleanPCR kit is an efficient PCR and Next Gen library prep clean up system based on paramagnetic beads technology, providing an efficient purification of PCR amplicons. With its simple, three-step protocol, CleanPCR removes salts, primers, primer-dimers, dNTPs, while DNA fragments are selectively bound to the magnetic particles; and highly purified DNA is eluted with low salt elution buffer or water and can be used directly for downstream applications. The protocol can be adapted to your current liquid handling workstation (e.g. Beckman, Hamilton, Tecan, Caliper, Perkin Elmer, Agilent and Eppendorf) utilizing your current protocol as well as it can be performed manually.

Features:

- High recovery of amplicons greater than 100 bp
- Efficiently removes unincorporated dNTPs, primers, primer dimers and other contaminants
- Stable and high recovery of PCR products post-cleanup
- No centrifugation or filtration

Amplicons purified with CleanPCR system are ready to be used in the following applications:

- PCR
- Mutation detection and Genotyping
- Sequencing (Sanger and Next Generation)
- Fragment Analysis
- Microarrays
- Restriction enzyme clean up
- Cloning

Kit Contents and Materials

Kit Contents:

Product Number	Description	Number of Reactions	Storage Conditions
CPCR-0005	CleanPCR – 5 mL	277 *	4-8^{0}C DO NOT FREEZE
CPCR-0050	CleanPCR – 50 mL	2.777 *	
CPCR-0500	CleanPCR – 500 mL	27.777 *	

* Number of reactions is based on a typical 10 µL PCR reaction volume.
Volume of CleanPCR to be used per reaction = 1.8x the sample volume.

Materials Supplied in the CleanPCR kit:
CleanPCR magnetic particle solution

Materials and Equipment to be supplied by User:

- 96-well PCR plate containing PCR samples (up to 50 µL/well)
- Magnetic separation device, recommended Clean Magnet Plate 96-Well RN50 (Part# CMAG-RN50)
- Multichannel pipettor
- Multichannel Disposable Reservoirs
- 96-well microplate for elution
- 70% ethanol (freshly prepared from non-denatured alcohol)
- Elution Buffer (10mM TRIS-HCL pH 8.0)

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CleanPCR - 96-well Plate Protocol

1. Shake the CleanPCR reagent thoroughly too fully resuspend the magnetic particles prior to usage.

2. Measure the PCR sample(s) reaction volume in the wells of the 96-well plate. Determine if transferring the sample(s) to a processing plate is required. If necessary, transfer the PCR reactions to a 96-well microplate.

   

   Note: If the PCR reaction volume * 2.8 exceeds the volume of the PCR plate, a transfer to a 300 µl round bottom plate is required.

3. Add 1.8x the reaction volume of CleanPCR to each well.

PCR Reaction Volume (µL)	CleanPCR (µL)
10	18
20	36
50	90

4. Incubate at room temperature for 5 minutes.

5. Place the plate on a magnetic separation device to magnetize the CleanPCR particles. Incubate at room temperature until the CleanPCR is completely cleared from solution.

6. Aspirate and discard the cleared supernatant. Do not disturb the CleanPCR particles.

7. Add 200 µL 70% ethanol to each well.

8. Incubate at room temperature for 1 minute. It is not necessary to resuspend the CleanPCR particles.

9. Aspirate and discard the cleared supernatant. Do not disturb the CleanPCR.

10. Repeat Steps 9-11 for a second 70% ethanol wash step.

11. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the CleanPCR particles. Remove any residue liquid with a pipet.

    

    Note: It is important to dry the CleanPCR particles before elution. Residual ethanol may interfere with downstream applications.

12. Remove the plate from magnetic separation device.

13. Add 30-40 µL Elution Buffer (not provided) to each well.

14. Pipet up and down 20 times or vortex for 30 seconds.

15. Incubate at room temperature for 2-3 minutes.

16. Place the plate on a magnetic separation device to magnetize the CleanPCR particles. Incubate at room temperature until the CleanPCR particles are completely cleared from solution.

17. Transfer the cleared supernatant containing purified DNA to a new 96-well microplate and seal with non-permeable sealing film.

18. Store the plate at 2-8°C if storage is only for a few days. For long-term storage, samples should be kept at -20°C.

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CleanPCR - 384-well Plate Protocol

1. Shake the CleanPCR reagent thoroughly too fully resuspend the magnetic particles prior to usage.

2. Place the 384-well PCR plate on the bench and measure the volume of the PCR reaction. Transfer the sample to a skirted 384-well PCR plate.

3. Add 1.8x the sample volume of CleanPCR reagent to each well.

PCR Reaction Volume (µL)	CleanPCR (µL)
5	9
7	12,6
10	18

4. Pipet up and down 5-10 times or vortex for 30 seconds.

5. Incubate at room temperature for 5 minutes.

6. Place the plate on a magnetic separation device to magnetize the CleanPCR particles. Incubate at room temperature until the CleanPCR particles are completely cleared from solution.

7. Aspirate and discard the cleared supernatant. Do not disturb the CleanPCR particles.

8. Add 30 µL 70% ethanol to each well.

9. Incubate at room temperature for 1 minute. It is not necessary to resuspend the CleanPCR particles.

10. Aspirate and discard the cleared supernatant. Do not disturb the CleanPCR particles.

11. Repeat Steps 8-10 for a second 70% ethanol wash step.

12. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the CleanPCR particles. Remove any residue liquid with a pipet.

Note: It is important to dry the CleanPCR particles before elution. Residual ethanol may interfere with downstream applications.

13. Remove the plate from magnetic separation device.

14. Add 30 µL Elution Buffer (not provided) to each well.

15. Pipet up and down 20 times or vortex for 30 seconds.

16. Incubate at room temperature for 2-3 minutes.

17. Place the plate on a magnetic separation device to magnetize the CleanPCR. Incubate at room temperature until the CleanPCR is completely cleared from solution.

18. Transfer the cleared supernatant containing purified DNA to a new 384-well microplate and seal with non-permeable sealing film.

19. Store the plate at 2-8°C if storage is only for a few days. For long-term storage, samples should be kept at -20°C.

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Trouble Shooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact your local distributor.

Possible Problems and Suggestions

Problem	Cause	Solution
Low yield	Low PCR product yield	Increase the number amplification cycles for PCR
	Smaller PCR product size	Small PCR fragments normally give lower yield.
	Ethanol residue	During the drying step, remove any liquid from bottom of the well
	Particle loss during the procedure	Increase magnetization time. Aspirate slowly.
	DNA remains bound to beads	Increase elution volume
	Incomplete resuspension of the particles during elution	Vortex or pipet up and down to fully resuspend the particles.
Primer carryover	Insufficient wash of the particles	Wash the beads one more time with 70% ethanol.
Non-specific amplification products were not removed	The size of the non-specific amplification products are larger than 100 bp	Non-specific amplification products larger than 100 bp are not efficiently removed from PCR products.
Problems in downstream applications	Salt carryover	70% ethanol must be stored at room temperature.
	Ethanol carryover	Ensure the beads are completely dried before elution.

Ordering Information

Contact your local distributor to order.

Product	Part Number
CleanPCR (5 mL)	CPCR-0005
CleanPCR (50 mL)	CPCR-0050
CleanPCR (500 mL)	CPCR-0500

Product	Part Number
Clean Magnet Plate 96-Well RN50	CMAG-RN50

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